AMENDMENT TO THE SPECIFICATION

Please add the following Paragraph on Page 1 after the Title of **USE OF LABELLED CCK-B RECEPTOR LIGANDS FOR THE DETECTION AND LOCALIZATION OF MALIGNANT HUMAN TUMOURS** as the first Section in the Patent Application:

APPLICATION CROSS-REFERENCES

This application is a continuation of U.S. Patent Application No. 09/125,823, filed January 19, 1999, now abandoned, and claims priority of International Application No. PCT/US97/03056 filed February 27, 1997 and published in English. This application also claims priority of European Patent Application No. 96200498.2, filed February 27, 1996.

Please replace paragraph [0005] on Page 2, Lines 19-37 and Page 3, Lines1-21 with the following amended paragraph:

The above-defined objective can be achieved, according to the present invention, by a method of detecting and localizing malignant tumours and their metastases in tissues, which in healthy condition do not contain disturbing quantities of CCK-receptors, in the body of a human being, which comprises (i) administering to said being a composition comprising, in a quantity sufficient for external imaging, a peptide derived from a compound of the general formula

H - $(Xaa)_n$ - $(Xbb)_m$ - Tyr - Xcc - Gly - Trp - Xdd - Asp - Phe - R_2 (I) (SEQ ID NO:27)

5

or an acid amide thereof, formed between a free NH2-group of an amino acid moiety and R_1COOH, wherein

R_1 is a (C_1-C_3) alkanoyl group, an arylcarbonyl group, or an aryl-

(C_1-C_3)alkanoyl group;

or a lactam thereof, formed between a free NH_2 group of an amino acid moiety and a free CO_2H

group of another amino acid moiety; or a conjugate thereof with avidin or biotin;

wherein:

$(Xaa)_n$ stands for 0 to 25 amino acid moieties which are equal or different and are selected from Ala, Leu, Asn, Dpr, Gln, Glu, Ser, Ile, Met, His, Asp, Lys, Gly, Thr, Pro, Pyr, Arg, Tyr. Trp, Val and Phe;

m = 0 or 1;

Xbb is Asp, Dpr, Glu or Pyr, with the proviso that Xbb can only be Pyr when n = 0;

Xcc is Met, Leu or Nle;

Xdd is Met, Leu or Nle; and

R_2 is a hydroxy group, an acetoxy group or an amino group;

said peptide being labelled with (a) a radioactive metal isotope selected from the group consisting of ^{99m}Tc, ^{203}Pb, ^{67}Ga, ^{68}Ga, ^{72}As, ^{111}In, ^{113m}In, ^{97}Ru, ^{62}Cu, ^{64}Cu, ^{52}Fe, ^{52m}Mn and ^{51}Cr, or (b) with a paramagnetic metal atom selected from the group consisting of Cr, Mn, Fe, Co, Ni, Cu, Pr, Nd, Sm, Yb, Gd, Tb, Dy, Ho and Er, or (c) with a radioactive halogen isotope, selected from ^{123}I, ^{131}I, ^{75}Br, ^{76}Br, ^{77}Br and ^{62}Br, and thereupon (ii) subjecting said being to external imaging, by radioactive scanning or by magnetic resonance imaging, to determine the targeted sites in the body of said being in relation to the background activity, in order to allow detection and localization of said tumours in the body.

Please replace paragraph [0013] on Page 6, Lines 12-37 and replace with the following amended paragraph:

Suitable examples of the above-defined peptides, which after labelling can be used in the method of the invention, are unsulfated CCK_7 and the corresponding CCK_8, CCK_9 and CCK_{10}-analogs. In formulas:

(1) unsulfated CCK_7 (= Tyr^{27}- CCK (27-33): H-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2 (SEQ ID NO: 1)

(2) unsulfated CCK_8 (= Tyr^{27}-CCK (26-33): H-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2 (SEQ ID NO:2)

(3) unsulfated CCK_8-analog 1 (=Tyr^{27},$Nle^{28,31}$-CCK (26-33)): H-Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (SEQ ID NO:3)

(4) unsulfated CCK_8-analog 2 (= $DAsp^{26}$,Tyr^{27},$Nle^{28,31}$-CCK (26-33)): H-DAsp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (SEQ ID NO:4)

(5) unsulfated CCK_8-analog 3 (= $DAsp^{26}$,Tyr^{27}-CCK (26-33)): H-DAsp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2 (SEQ ID NO:5)

(6) unsulfated CCK_8-analog 4 (= Dpr^{26},Tyr^{27},$Nle^{28,31}$-CCK (26-33)): H-DprTyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (SEQ ID NO:6)

(7) unsulfated CCK_8-analog 5 (= Tyr^{27},Thr^{28},Nle^{31}-CCK (26-33)): H-Asp-Tyr-Thr-Gly-Trp-Nle-Asp-Phe-NH_2 (SEQ ID NO:7)

(8) unsulfated CCK_9-analog 1 (= Tyr^{27},$Nle^{28,31}$-CCK (25-33)): H-Arg-AspTyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (SEQ ID NO:8)

(9) unsulfated CCK_9-analog 2 (= Tyr^{27},Thr^{20},Nle^{31}-CCK (25-33)): H-Arg-Asp-Tyr-Thr-Gly-Trp-Nle-Asp-Phe-NH^2 (SEQ ID NO:9)

(10) unsulfated CCK_{10}-analog 1 (=Tyr^{27},$Nle^{28,31}$-CCK (24-33)): H-Tyr-Gly-Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (SEQ ID NO:10)

(11) unsulfated CCK_{10}-analog 2 (= $DTyr^{24}$,Tyr^{27},$Nle^{28,31}$-CCK (24-33)): H-DTyr-Gly-Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (SEQ ID NO:11)

Please replace Paragraph [0021] on Page 11, Lines 9-17 with the following amended paragraph:

The invention also relates to a pharmaceutical composition to be used for the method of intraoperatively detecting and localizing malignant tumours as mentioned above, comprising in addition to a pharmaceutically acceptable carrier material and, if desired, at least one pharmaceutically acceptable adjuvant, as the active substance, in a quantity sufficient for intraoperatively detecting and localizing malignant tumours, a peptide selected from the group consisting of [^{125}I-D-Tyr]-Gly-Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (SEQ ID NO:13) and D-

Tyr-Gly-Asp [^{125}I-Tyr]-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (SEQ ID NO:14).

Please replace Paragraph [0023] on Page 11, Lines 26-29 with the following amended paragraph:

The invention also relates to the compounds [^{125}I-D-TyrJ -Gly-Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (SEQ ID NO:14) and D-Tyr-Gly-Asp-[^{125}I-Tyr]-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (SEQ ID NO:14) especially to be used in the method of intraoperatively detecting and localizing malignant tumours

Please add the following new paragraph on Page 15, after Line 13 and before Line 15 after Paragraph [0032]:

BRIEF DESCRIPTION OF THE DRAWINGS

Figures lA-C show displacement curves of [^{125}I]-CCK-l0 analog (compound 15) binding to tissue sections from three different tumours. Figure 1 A shows results with medullary thyroid carcinoma (MTC). Figure lB shows results with small cell lung carcinoma (SCLC). Figure lC shows results with gastro entero pancreatic tumour (GEP-Tu).

Figures 2A-B show displacement curves of [^{125}I]-CCK-10 analog (compound 15) binding to tissues from two different tumours. Figure 2A shows results with medullary thyroid carcinoma (MTC). Figure 2B shows results with meningioma.

Figure 3 shows displacement curves of [^{125}I]-CCK-10 analog (compound 15) binding to tissues from medullary thyroid carcinoma (MTC).

Figure 4 shows displacement curves of [^{125}I]-desulfated-CCK-10 (compound 13 or 14) binding to tissue sections from medullary thyroid carcinoma (MTC).

Figures 5A-B show autoradiograms of the binding of ^{125}I desulfated CCK-10 ligand to CCK-B receptors in medullary thyroid carcinoma (MTC). Figure 5A is an autoradiogram showing total binding of the ligand. Figure SB is an autoradiogram showing non-specific binding in the presence of 10^{-6} M desulfated CCK-10 analog.

EXAMPLES

Please replace paragraph [0033] on Page 15, Lines 15-25 and replace with the following amended paragraph:

Example 1. Preparation of Compound 12

The peptide DTyr-Gly-Asp-Tyr- Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (SEQ ID NO:12) (Compound 12) is synthesised using the Chiron-Multipin Synthesis Technology (Geysen et al., Proc. Natl. Acad. Sci. 1964, 81, 3998-4002; Geysen et al., *J. Immunol. Methods 1987*, 102, 259-274). The peptide is analysed with HPLC (Merck Lichrospher 100 RP-18, 250*4mm, Gradient elution (A: 0.1% orthophosphoric acid in water; B: 0.1% orthophosphoric acid in 90% acetonitrile; 0-67% B in 15 minutes; Flow rate 1.5 ml; Detection wavelength 214 ml; Detection wavelength 214 nm) and by Ion Spray Mass Spectrometry.

Results: purity (HPLC) : 97.8%
Mw (IS-MS) : 1247.3 (calculated 1247.4)

Please replace paragraph [0036] on Page 16, Lines 27-33 and Page 17, Lines 1-19 and replace with the following amended paragraph:

The following CCK derivatives were synthesized based on the above general procedure. The analyses were performed on a Finnigan TSQ-700 Triple Quad Mass Spectrometer with an Atmospheric Pressure Ionization interface. The samples were introduced by flow injection analysis into acetonitrile/water with 0.1% trifluoroacetic acid. Electrospray ionization was the mode of ionization employed and the instrument was run in positive ion mode.

1. DTPA-Tyr^{27}-CCK (26-33): DTPA-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2 (SEQ ID NO:19)

(Compound 19). Molecular Weight, Calculated: 1437.5, Found: 1438.8 (M^++1).

2. DTPA-Tyr^{27},$Nle^{28,30}$-CCK(26-33): DTPA-Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (SEQ ID

NO:20) (Compound 20) . Molecular Weight, Calculated: 1401.6, Found: 1402.8 (M^++l)

3. DTPA-DAsp^{26},Tyr^{27},Nle^{28,31}-CCK(26-33): DTPA-DAsp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (SEQ ID NO:21) (Compound 21) . Molecular Weight, Calculated:1401.6, Found: 1402.8 (M^++1).

4. DTPA-DAsp^{26},Tyr^{27},-CCK(26-33): DTPA-DAsp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2 (SEQ ID NO:22) (Compound 22) . Molecular Weight, Calculated: 1437.5, Found: 1438.8 (M^++1).

5. Dpr^{26}(β-DTPA)-Tyr^{27},Nle^{28,31}-CCK(26-33): Dpr(β-DTPA)-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (SEQ ID NO:23) (Compound 23) . Molecular Weight, Calculated: 1372.6, Found: 1373.7 (M^++1).

6. DTPA-Tyr^{27},Thr^{28},Nle^{31}-CCK(26-33): DTPA-Asp-Tyr-Thr-Gly-Trp-Nle-Asp-Phe-NH_2 (SEQ ID NO:24) (Compound 24). Molecular Weight, Calculated:1389.5, Found: 1390.5 (M^++1).

Please replace paragraph [0037] on Page 17, Lines 20-36 and replace with the following amended paragraph:

Example 3. Preparation of ^{115}In labelled Compounds 25 and 26.

Peptides are dissolved in 5mM $NaHCO_3$ at a concentration of 2.0 mg/ml. Labelling conditions are performed using a 1.5:1.0 molar ratio of $^{115}In^{3+}$ ~~(as $InCl^3$ to peptide.~~ (as $InCl^3$ to peptide).

Labelling procedure:

To 50 µl of peptide solution (100 µg peptide, 71.4 nmol) is added 23.7 µl (107.1 nmol)of a $^{115}InCl_3$ in 0.05N HC1 (1.0 mg/ml) solution. Water is added to bring the final volume of the reaction to 200 µl. After 15 minutes at room temperature the solution is frozen and subsequently lyophilized to dryness. Dried ^{115}In complexed peptide is re-dissolved in 10 mM $NaHCO_3$ and analyzed by reversed phase HPLC and by Mass Spectroscopy.

With the above mentioned labelling procedure compounds 25 (^{115}In-DTPA-Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2) (SEQ ID NO:25) and 26 (^{115}In-DTPA-DAsp-Tyr-Nle-Gly-Trp-Nle-Asp-

Phe-NH_2) (SEQ ID NO:26) were prepared. HPLC analysis indicated that the peptides were > 99% complexed with ^{115}In. Mass spectroscopy analysis yielded the expected molecular weight.

Please replace paragraph [0038] on Page 18, Lines 1-37 and Page 19, Lines 1-29 and replace with the following amended paragraph:

Example 4. Receptor affinity studies with unlabelled compounds.
Compounds 15, 16 and 18 are only used by comparison and are not in the scope of the present invention. Receptor autoradiography is performed on 10- and 20-µm thick cryostat sections of the various tumour samples, as described by Reubi et al. (Cancer Res. 1990, 50, 5969-5977) Unlabelled CCK-8 (= Asp-Tyr(SO_3H) -Met-Gly-Trp-Met-Asp-Phe-NH_2, (SEQ ID NO:16) compound 16) and un-labelled desulfated CCK-8 (= Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2, (SEQ ID NO:17) compound 17; are obtained from Bachem AG, Bubendorf, Switzerland. Unlabelled CCK-10 analog (= D-Tyr-Gly-Asp-Tyr (SO_3H) -Nle-Gly-Trp-Nle-Asp-Phe-NH_2, (SEQ ID NO:18) compound 18) is obtained from Research Plus Laboratories, Bayonne, NJ, USA.
^{125}I-labelled peptides are prepared via the chloramine T ionization procedure, according to procedures as reported earlier by Greenwood et al. (Biochemical Journal 1963, 89, 114-123). The [^{125}I-D-Tyr^{24}],Nle^{28,31}-CCK 24-33 labelled peptide 15 (= [^{125}I-D-Tyr] Gly-Asp-Tyr (SO_3H)-Nle-Gly-Trp-Nle-Asp-Phe-NH_2) (SEQ ID NO:15) is separated by HPLC, using a reverse phase RC_{18} column and butane-sulphonic acid as the eluent. The mono-^{125} iodinated compound is eluted as single peak from the HPLC and analysed by mass-spectrometry. Specific activity: 2000 Ci/mmol. The tissues are cut on a cryostat, mounted on microscope slides, and then stored at – 20° C for at least 3 days to improve adhesion of the tissue to the slide. The slide-mounted tissue sections are allowed to reach room temperature and are preincubated in 50 mmol/l Tris-HCl, 130 mmol/l NaCl, 4.7 mmol/l KCl, 5 mmol/l $MgCl_2$, 1 mmol/1 ethylene glycol-bi(β-aminoethylether)-N,N,N',N'-tetraacetic acid, and 0.5% bovine serum albumin, pH 7.4 (preincubation solution), for 30 min. at 25°C. The slides are then incubated in a solution containing the same medium as the preincubation solution except the bovine serum albumin is omitted, and the following compounds are added: 20000 dpm/100 µl of ^{125}I-CCK, 0.025%

bacitracin, 1 mmol/1 dithiothreitol, 2μg/ml chymostatin, and 4μg/ml leupeptin, pH = 6.5. The slides are incubated at room temperature with the radioligand for 150 min., as described by Mantyh et al. (Gasteroenterology 1994, 107, 1019-30) . To estimate non-specific binding, paired serial sections are incubated as described above, except that CCK-8 (sulfated) is added to the incubation medium. After the incubation, the slides are rinsed with four washes of 30 sec each in ice-cold preincubation solution, pH 7.4, dipped in ice-cold water, and then quickly dried in a refrigerator under a stream of cold air. The sections are subsequently exposed to a ^{3}H-Ultrofilm for 1 week, to detect the precise location of the radioactivity.

In all tumours, displacement experiments using successive sections of a tumour are performed with increasing concentrations of various biologically active or inactive peptides (see the above-mentioned publication by Reubi et al.)). In comparison with sulfated CCK, unsulfated CCK, as well as somatostatin are used.

The figure 1 attached shows displacement curves of [^{125}I]-CCK-10 analog (compound 15) binding to tissue sections from three different tumours: A = medullary thyroid carcinoma (MTC) and B = small cell lung carcinoma (SCLC) and C = Gastro entero pancreatic tumour (GEP-Tu). Tissue sections are incubated with 20,000 cpm/100μl [^{125}I]-CCK-10 and increasing concentrations of unlabelled CCK-8 (unsulfated) (▲), CCK-8 (sulfated) (●), or somastotatin (o). Each point represents the optical density of binding measured in the tumour area. Non-specific binding is subtracted from all values. In all cases, complete displacement of the ligand is achieved by sulfated CCK and unsulfated CCK is inactive in GEP-Tu, whereas somastotatin is inactive in the nanomolar range for all three types of tumours.

This experiment shows that MTC and SCLC, expressing CCK-B tumours and that GEP-Tu, expressing CCK-A receptors, can respectively be detected with both sulfated and unsulfated radiolabelled CCK or with sulfated radiolabelled CCK only. Therefore it can be concluded that tumours expressing CCK-B receptors can selectively be detected with unsulfated radiolabelled CCK without disturbing effects of CCK-A receptor expressing tissues or tumours.

Please replace paragraph [0042] on Page 21, Lines 5-29 and replace with the following amended paragraph:

Example 7

The experiments are performed as described in Example 4. Instead of the [^{125}I]-CCK-10 analog the desulfated [^{125}I]-CCK-10 compound (= ^{125}I[D-Tyr-Gly-Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2]) (SEQ ID NO:13) is prepared by iodination of compound 12 as described in Example 1. The two monoiodinated compounds 13 and 14 obtained after iodination are separated by HPLC, using a reverse phase RC_{18} column and butane-sulphonic acid as the eluent. The two mono-^{125}-iodinated compounds are eluted as a single peak from the HPLC and are analysed by mass-spectrometry.

The figure 4 attached shows displacement curves of [^{125}I]-desulfated CCK-10 binding (compound 13 or 14) to tissue sections from medullary thyroid carcinoma (MTC). Tissue sections are incubated with 20,000 cpm/100µl] [^{125}I]-desulfated-CCK-10 and increasing concentrations of compound 17 (CCK-8 (unsulfated)) (▲), compound 16 (CCK-B (sulfated)) (●), CCK-10 (unsulfated) (▼) or somastotatin (o). Each point represents the optical density of binding measured in the tumour area. Non-specific binding is subtracted from all values. In all cases, complete displacement of the ligand is achieved by sulfated and unsulfated CCK, whereas somastotatin is inactive in the nanomolar range. The two different mono ^{125}I-iodinated compounds appear to have the same affinity. Figure 5 attached shows the autoradiogram of the binding of ^{125}I desulfated CCK-10 ligand to CCK-B receptors in MTC. A = Autoradiogram showing total binding of the ligand; B = Autoradiogram showing non-specific binding (in the presence of 10^{-6} desulfated CCK10 analog).

Please delete the Sequence Listing of Pages 22-48 and add the enclosed Sequence Listing (Pages 1-16) as an attachment to the application.